Journal of Clinical Trials
ISSN: 2167-0870

Fan et al., J Clin Trials 2016, 6:6
DOI: 10.4172/2167-0870.1000293

Protocol Open Access

Effect of Dietary Nitrate Supplementation on Blood Pressure Variability and Vascular Function in High-Risk Transient Ischemic Attack Patients

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Received date: November 06, 2016; **Accepted date:** November 25, 2016; **Published date:** December 05, 2016

Abstract

Background: Patients presenting with a transient ischemic attack (TIA) are at high risk of stroke despite current treatments. Elevated blood pressure variability (BPV) and vascular dysfunction are known to increase the risk of stroke in TIA patients. Therefore, improving these hemodynamic parameters could help reduce stroke incidences in these patients.

Aim: The proposed study will investigate the efficacy of dietary nitrate supplementation on cardiovascular and cerebrovascular hemodynamics in patients recently diagnosed with TIA.

Methods: This study is a randomized, placebo-controlled, parallel group clinical trial, with patient recruitment based on strict inclusion/exclusion criteria. Newly diagnosed patients who present within 48 h of symptom onset will be assessed to ascertain their post-TIA, pre-treatment baseline cardiovascular and cerebrovascular parameters. These will include: beat-to-beat BPV, cerebrovascular CO_2 reactivity and cerebral autoregulation (indices of cerebrovascular health), brachial artery diameter, central and peripheral blood pressures, vascular risk factors (i.e. resting blood pressure), and plasma nitrate/nitrite concentration. Following pre-treatment assessment, participants will be randomized to take either 7-day dietary nitrate supplementation (sodium nitrate in capsules, 10 mg/kg/day) or 7-day placebo. An identical follow-up assessment will be implemented post-intervention.

Conclusion: This study will lay the foundation for clinical trials to assess the therapeutic potential of dietary nitrate supplementation as a secondary strategy for stroke prevention in high-risk patients.

Keywords: Stroke; Transient ischemic attack; Cerebral blood flow; Cerebral oxygenation; Dietary nitrate supplementation

Introduction

Ischemic stroke is a devastating disease which accounts for ~70% of all strokes worldwide [1], with limited secondary prevention strategies. Transient ischemic attack (TIA) precedes around one-quarter of all ischemic strokes [2]. Despite current treatments, ~7-23% of TIA patients experience recurrent TIAs or ischemic strokes within the first week of symptom onset [2-5]. It is known that increased systolic blood pressure variability (BPV) and vascular dysfunction are associated with early stroke recurrence after ischemic stroke and TIA [6-8]. But there are currently no therapeutic treatments to dampen BPV and improve vascular function in this high-risk patient group.

Nitric oxide (NO) is a potent regulator of vascular tone, which can be produced from a number of different sources [9,10]. NO production by endothelial NOS (eNOS) plays a crucial role in cardio- and cerebrovascular hemodynamic regulation, and is neuroprotective following ischemic stroke [9,10]. In animal models of ischemic stroke, administration of NO donors or intra-arterial L-arginine increases eNOS activity and regional CBF, and reduces infarct volumes [11-13]. Basal NO release inhibits platelet and leukocyte aggregation, and reduces microvascular permeability [14-16]. Besides endogenous NO production by eNOS, the other major source of nitrate is from diet [17]. Dietary inorganic nitrate is reduced to nitrite within the saliva [18], and further reduced to NO by red blood cells [19]. In healthy populations, dietary nitrate improves cerebral blood flow (CBF) regulation [20], and abolishes hypoxia-induced endothelial dysfunction at high altitude [21]. Similarly, dietary nitrate supplementation has been shown to improve vascular function and carotid artery stiffness, and reduce systolic blood pressure (BP) in elderly populations with moderate cardiovascular risk [22,23]. Dietary nitrate could be a safe and effective strategy for dampening BPV and improving vascular function.

Currently, we know little about the hemodynamic effects of dietary nitrate supplementation on high-risk TIA patients. Establishing the biological effects of increased NO bioavailability on BPV and cerebrovascular function in TIA patients is a necessary first step towards an effective clinical translation of this potential therapeutic

strategy. The goal of the proposed study is to examine the role of NO bioavailability in BP and CBF regulation. We test the hypothesis that increasing NO bioavailability with dietary nitrate supplementation dampens BPV and improve cerebrovascular functions in TIA patients.

Patient population

All suspected TIA referrals will be reviewed by a neurologist at the Wellington Hospital and appropriate diagnostic tests undertaken (stroke classification, computed tomography scan, computed tomography angiography scan, magnetic resonance imaging, magnetic resonance angiography imaging, carotid ultrasound imaging, echocardiography, electrocardiogram) including the National Institutes of Health Stroke Scale (NIHSS) and modified Barthel Index.

Patients diagnosed with an acute TIA will be recruited within 48 hours of symptom onset. The TIA cohort is likely to benefit from dietary nitrate supplementation because these patients are at high risk of early or recurrent stroke, which is associated with BPV and vascular dysfunction [6-8]. Aside from nitrate supplementation, patients will be managed according to routine clinical practice. Inclusion and exclusion criteria are detailed in Table 1.

Inclusion criteria	Exclusion criteria
Individuals aged 40-85 diagnosed with TIA (with $ABCD_2$ score ≥ 4), after review by a neurologist at Wellington Hospital.	Individuals requiring supplementary oxygen
	Allergic to nitrates
	Unstable cardiac conditions or angina
	Uncontrolled diabetes mellitus
	Major medical conditions
	Significant cognitive impairment
	Immobility
	Age >85 years
	TIA symptom onset >48 h

Table 1: Inclusion and exclusion criteria.

Methods

Experimental design

This is a single-center, placebo-controlled, single-blinded, randomized, parallel group clinical trial. This study is designed to ensure the reflection of a potential real-life application of a dietary supplementation intervention for TIA patients following diagnosis (Figure 1). The patients will visit the laboratory on two occasions, which will consist of a pre-treatment assessment (visit one) and a follow-up assessment one week later (visit two). Once recruited, patients will undergo pre-treatment assessment as a part of their clinical assessment with the neurology department. Thereafter, patients will receive either nitrate supplementation (sodium nitrate) or placebo for seven days. An identical assessment will be performed at the follow-up experimental session.

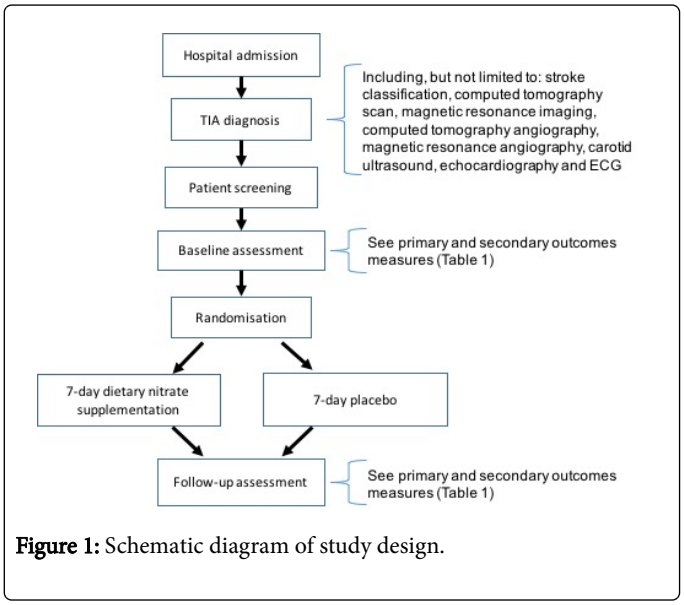

Figure 1: Schematic diagram of study design.

Dietary nitrate supplementation

Oral sodium nitrate capsules (10 mg/kg/day) will be ingested three times a day with each meal, for seven days. This dosage has been shown to elevate plasma nitrate (580%) and nitrite (180%) [24]. Those patients assigned to the placebo group will be given identical-looking capsules containing microcrystalline cellulose. All of the patients will be instructed to avoid using mouthwash during the intervention period as it has been shown to abolish the effect of dietary nitrate on plasma nitrite/nitrate levels [25].

Experimental procedures

Each experimental testing session will comprise of: i) 20 min instrumentation; ii) venous blood sample; iii) 10 min resting baseline; iv) CO_2 reactivity; and v) flow-mediated dilatation (FMD). All of the measurements will be conducted with the participants resting in a supine position. Primary and secondary outcomes are outlined in Table 2.

Dependent variable	Procedure/measures
Primary	
Beat-to-beat blood pressure variability	Finger photo plethysmography will be used to continuously monitor peripheral blood pressure
Cerebral autoregulation	Relationships between beat-to-beat blood pressure, middle cerebral artery blood velocity (MCAv) and cerebral oxygenation will be assessed using wavelet analysis.
Cerebral CO2 reactivity	MCAv will be assessed using Transcranial Doppler ultrasonography (TCD) during CO_2 breathing and voluntary hyperventilation to increase and decrease end-tidal partial pressure of CO_2 (+/-5 mmHg).

Flow-mediated dilatation	Brachial blood flow response will be assessed during, and post 5-min forearm occlusion.
Secondary	
Central & Peripheral blood pressures	Pulse wave analysis will be employed to assess central blood pressure, augmentation index and arterial stiffness.
Vascular risk factors	Resting systolic and diastolic blood pressure. Body weight and body mass index.
Plasma nitrate/nitrate concentration	Resting venous blood sample.

Table 2: Study outcomes measured at pre-treatment baseline and post-treatment follow-up.

CO2 reactivity test

Cerebrovascular function will be assessed using a dynamic CO_2 reactivity test previously described by Peebles et al. [26]. Guided by a metronome, the patients will be instructed to breath at a rate of 12 breaths/min throughout the CO_2 reactivity test. A CO_2 gas mixture (5% CO_2, 21% O_2 in nitrogen) will then be administered using a facemask, to progressively elevated the patient's partial pressure of end-tidal CO_2 ($PETCO_2$) to ~5 mmHg above their resting values over a ~120 s period. Following a 30 second recovery period, the patients will be instructed to increase their tidal volume to lower $PETCO_2$ to ~5 mmHg below their resting value for ~120 s.

Flow-mediated dilatation

Endothelial function will be assessed by measuring flow-mediated dilatation of the left branchial artery. In brief, the left brachial artery will be visualized 2 to 10 cm above the elbow with a 10-Mhz Duplex Doppler ultrasound system (T3200, Terason, Burlington, MA, USA). 1 minute after acquisition of the baseline diameter, the forearm cuff immediately distal to the elbow will be inflated to 200 mmHg for 5 minutes. The arterial diameter will be continuously recorded for 1 minute prior to and 3 minutes following the cuff release.

Measurements

Plasma nitrate/nitrite concentration: A venous blood sample will be drawn from a catheter in the forearm antecubital vein. The samples will be placed in ice and subsequently centrifuged at 2000 rpm for 10 min (Sigma 2-4 centrifuge, Sigma, Osterode am Harz, Germany). The plasma will then be removed and frozen at -80°C pending analysis of plasma nitrate/nitrite concentration.

Cerebrovascular parameters: Middle cerebral artery blood flow velocity (MCAv, as an index of cerebral perfusion) will be measured bilaterally from the middle cerebral arteries using a 2-MHz pulsed transcranial Doppler ultrasound system (ST3, Spencer technology, Seattle, USA). The ultrasound probes will be positioned over the temporal windows and held firmly in place with an adjustable headband (Marc 600 Head Frame, Spencer Technology, Seattle, USA). The signals will be obtained by first locating the bifurcation of the middle and anterior cerebral arteries; the angle and depth of insonation will then be adjusted to obtain measurements from the MCA. The insonation depth and the velocity of MCA signals will be recorded and compared to ensure within-subject repeatability of MCAv measurements between visits. Cerebral tissue oxygenation in the bilateral prefrontal cortex will be assessed by monitoring changes in total-, oxy-, deoxy-, delta-hemoglobin concentrations and cerebral O_2 saturation obtained with spatially resolved, continuous wave Near-infrared spectroscopy (NIRS, Oxiplex TS, ISS Inc., Champaign, IL, USS).

Cardiorespiratory parameters: Beat-to-beat means arterial BP will be monitored using finger plethysmography (Vinometer® MIDI, Finapress Medical Systems, Amsterdam, Netherlands). In addition, peripheral and central blood pressures will be estimated using Pulse Waveform analysis (BP+, Uscom, Sydney, Australia). A three-lead electrocardiogram will be used to determine heart rate (ML132 bio amp, ADInstruments, Dunedin, New Zealand). Partial pressure of end-tidal oxygen and carbon dioxide will be sampled using a plastic nasal cannula inserted into the left nostril, and analyzed using a fast-responding gas analyzer (ML206 gas analyzer, ADInstruments, Dunedin, New Zealand). Prior to each experimental session, the gas analyzer will be calibrated using precision gas mixture of know O_2 and CO_2 concentrations.

Sample size estimate

The sample size estimate was based on published [20,27] and unpublished data from our laboratory, which assessed the effects of dietary nitrate on cerebrovascular function. These were used to estimate a physiologically relevant improvement in cerebrovascular CO_2 reactivity of 16% between the two randomized groups. Assuming that dietary nitrate supplementation can improve cerebrovascular function by a similar extent, a total sample size of 34 patients is needed (i.e. 17 patients per group). Assuming a participant drop-out rate of 10%, 38 TIA patients will be recruited into this study. This sample size would provide >80% power to detect a moderate effect size that corresponds to a~16% difference in cerebrovascular function between treatment and placebo, assuming a standard deviation of 0.65%/mmHg at a two-tailed significance level of 0.05.

Data analysis

Participant compliance and adherence to the assessment and intervention will be monitored throughout the study. Baseline characteristics of the two study groups will be described by means and standard deviations. The key independent factor is treatment status, and we will control for the time of day which is a potential confounding factor. Mixed model linear regression (unstructured, different variance and correlation between measurements assumed) (IBM® SPSS® Statistics version 23, IBM® Corporation, Armonk, NY, USA) will be performed to evaluate the main effects of treatment (placebo *vs.* treatment) and time (baseline and follow-up) on primary and secondary outcomes. Post-hoc tests will be performed using the Holm-Sidak adjustment for multiple comparisons.

Current status of the trial

The study was started in September 2015 and the estimated completion date is December 2017. As of November 2016, 21 patients have been recruited.

Discussion

TIA is defined as a 'transient episode of neurological dysfunction caused by cerebral, spinal, or retinal ischemia without acute infarction, as assessed using available imaging' [28]. Vascular dysfunction such as carotid stenosis is associated with early recurrences after ischemic stroke, and is a risk marker for recurrent stroke after a TIA [6-8]. The overarching goal of the proposed study is to assess the therapeutic effects of increasing NO bioavailability on BPV and vascular function following a TIA event.

Nitric oxide in neuroprotection

There are two possible pathways by which increasing NO bioavailability may confer protection against stroke (Figure 2). First, by improving perfusion to penumbral tissue and neuronal survival, which reduces infarct volume and improves functional outcome? In mouse, rat and sheep models of ischemic stroke, administration of L-arginine, NO donors, and NO inhalation has been shown to reduce infarct volumes and improve neurological functions [11-13,29]. These authors attributed these improvements in stroke outcome to dilation of cerebral arterioles in the ischemic penumbra, thereby improving blood flow to under-perfused regions of the brain. Meanwhile, a recent multi-center clinical trial failed to observe any improvements in functional outcome in acute stroke patients following 7-days of transdermal glyceryl trinitrate treatment [30]. However, since the transdermal glyceryl trinitrate has no effect on cerebral perfusion following stroke [31-33], it is likely that transdermal nitrate administration did not improve penumbral perfusion. In contrast, dietary nitrate has been shown to modulate CBF response to visual stimulation in healthy participants [20,27]. The authors attributed these findings to an enhanced neurovascular coupling associated with dietary nitrate supplementation.

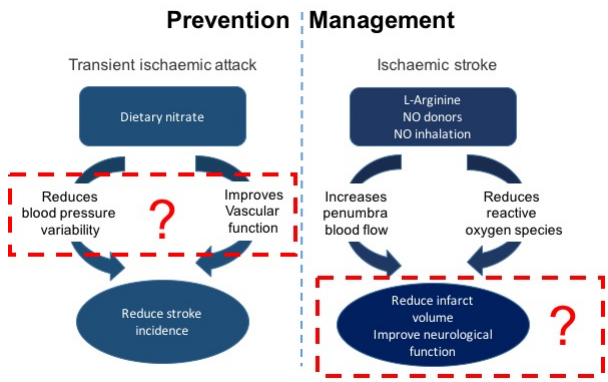

Figure 2: Summary of therapeutic strategies of increasing NO bioavailability.

In stroke management, increasing NO bioavailability with L-arginine, NO donors and NO inhalation has been shown to reduce infarct volume by improving penumbral blood flow and reducing reactive oxygen species in animal models of ischemic stroke [11-13,29]. In contrast, large multi-center clinical trials did not observe improvements in functional outcome with transdermal glyceryl trinitrate patches [30]. This study aims to explore the effects of increasing NO bioavailability on blood pressure variability and vascular function in TIA patients. Findings from this study will be the crucial first steps towards translational studies into the use of dietary nitrate as a secondary preventive strategy for stroke.

Second, increasing NO bioavailability could reduce the risk of stroke in high-risk populations via dampening BPV and improving endothelial function. In patients with cardiovascular risk factors and hypertension, dietary nitrate improves peripheral vascular function and aortic stiffness, and reduces resting BP [22,23]. Similarly, dietary nitrate supplementation improves vascular function and lowers systolic BP in healthy populations [21,27,34]. Collectively, these studies demonstrate a therapeutic benefit of dietary nitrate on vascular function in both healthy and clinical populations. However, the effect of dietary nitrate on BPV remains unclear. The goal of this study is to assess the effect of dietary nitrate supplementation on BPV and cerebrovascular function during the 7-day period following a TIA, when the incidence of stroke is the highest [2,3]. Such intervention strategy could be used to both reduce the stroke risk in TIA patients and provide neuroprotection following acute stroke (Figure 2).

Dietary nitrate supplementation

Ingestion of dietary nitrate has been shown to elevate plasma nitrate and nitrite, and lower BP in a dose-dependent manner, with reduced BP observed following ingestion of ≥ 8.4 mmol of inorganic nitrate [35,36]. However, these studies found beetroot juice had greater potency in lowering BP compared to nitrate salt, presumably due to additional polyphenols and antioxidants in beetroot. Nevertheless, studies have reported improvement in vascular function and reduced BP ~3h post ingestion of nitrate salts (5-8 mmol) [21,37]. Similarly, 3-day sodium nitrate supplementation (0.1 mmol/kg/day) enhanced neurovascular coupling during visual stimulation [27]. Based on these findings, we expect the proposed dosage of nitrate supplementation (~9.0 mmol/day) will be sufficient to observe any BP and cerebrovascular hemodynamic effects after 7 days.

Hemodynamic variability

According to conventional wisdom, high BP (i.e. hypertension) is the biggest risk factor for stroke. However, a growing body of evidence suggesting that dramatic variation in BP is another important independent risk factor for stroke and poor neurological outcomes. Accentuated fluctuations in BP results in hypo- and hyper-perfusion insults to vital organs like the brain, which can destabilize cerebral tissue oxygenation and lead to blood-brain barrier breakdown [38]. We recently observed greater dynamic BPV, but not CBF variability, in TIA patients compared to healthy controls [39]. Meanwhile, others have reported visit-to-visit variability in systolic BP, independent of average resting systolic BP, to be a strong predictor of subsequent stroke in TIA patients [8]. Following acute ischemic stroke, augmented systolic BPV is associated with severe hemorrhagic transformation [40] and poor early outcomes [41]. Conversely, reduced successive variability of diastolic BP has been shown to be a predictor of favorable long-term outcome [42]. These findings indicate that accentuated BPV adversely increases the risk of stroke in TIA patients, and leads to poorer outcome following ischemic stroke. Dampening BPV should be one of the main focuses of therapeutic treatment in these clinical populations.

Whilst dietary nitrate supplementation has been previously shown to lower resting BP [22,23], its effects on BPV remains unknown.

Cerebrovascular function

Two of the most commonly used techniques to assess cerebral hemodynamic integrity in cerebrovascular research are cerebrovascular CO_2 reactivity and cerebral autoregulation (CA). Cerebrovascular CO_2 reactivity is the CBF's response to CO_2, and it represents the dilatory and constrictive capacity of the cerebral arterioles to CO_2. In the absence of major arterial stenosis, reduced cerebrovascular CO_2 reactivity is assumed to reflect increased stiffness of the arteriolar walls [43]. In clinical populations, low cerebrovascular CO_2 reactivity is a predictor for ischemic stroke and TIA in patients with severe carotid artery stenosis or occlusion [44-46]. Meanwhile, CA reflects the dynamic myogenic, neurological, and metabolic vascular responses to changes in perfusion pressure in order to maintain reasonably constant CBF [47]. Impairment in dynamic CA results in concurrent fluctuations in mean CBF with fluctuations in arterial BP, thereby increasing susceptibility of white matter damage during these BP fluctuations [48]. Further, CA impairment is associated with various subtypes of stroke [49,50] and carotid artery stenosis [51]. These findings implicate impaired cerebrovascular function in the development of stroke. Cerebrovascular indices such as cerebrovascular CO_2 reactivity and CA provide invaluable information on the therapeutic effects of dietary nitrate on cerebrovascular health.

Flow-mediated dilatation

FMD of the brachial artery is the most commonly used technique to study endothelial function in vivo [52]. This non-invasive, ultrasound-based method first described by Celermajer et al [53], involves the assessment of peripheral conduit artery diameter following a period of distal limb ischemia. FMD has been shown to correlate well with coronary artery endothelial function [53], and is an independent predictor of cardiovascular disease [54]. The principal mediator of FMD response is endothelium derived NO [55], and studies have consolidated the link between increases in flow, wall shear stress, eNOS expression and NO bioactivity [56]. Therefore, FMD is an ideal tool for assessing the effect of dietary nitrate supplementation on endothelial function.

Stroke is a devastating disease with limited acute therapeutic options to improve outcomes. The proposed human trial will be the first to determine whether dietary nitrate supplementation has any positive therapeutic effects on BPV and cerebrovascular function in high-risk TIA patients. The data generated from this study will lay the foundation for future clinical trials to assess the role of dietary nitrate supplementation on stroke prevention and BP management. Such supplementation would be very cost-effective to implement, readily available, and could result in major healthcare gains for a clinical population that is over-represented among disabled individuals worldwide.

Acknowledgements

This study is supported by the University of Otago Research grant and the University of Otago Wellington Grant-in-aid, and the New Zealand Heart Foundation Small Project Grant. Dr Fan is supported by a New Zealand Heart Foundation Fellowship. The authors would like to thank Prof Mark Gladwin for his assistance in the study design.

Ethical approval and informed consent

This study has received ethical approval from the New Zealand Health and Disability Ethics Committee. It has also been registered with the Australian and New Zealand Clinical Trials Registry: ACTRN12616000086460. All participants will be informed regarding the procedures of the study, and written informed consent will be obtained prior to participation.

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